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Supplemental Information

Heterogeneous Responses of Hematopoietic Stem Cells to Inflammatory Stimuli Are Altered with Age

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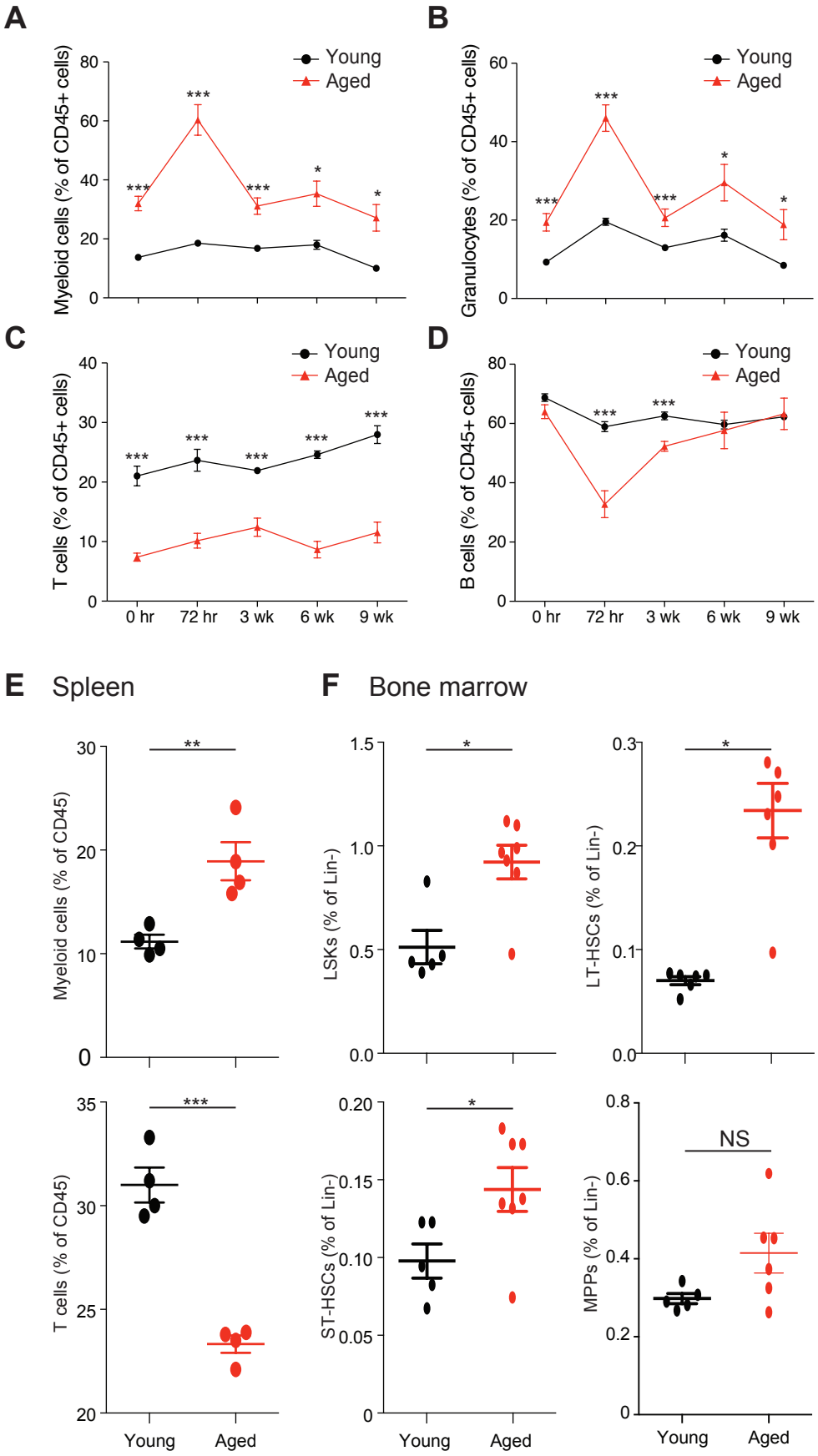

Figure S1. Aged mice challenged with LPS demonstrate increased myeloid output and an increased frequency of bone marrow HSPCs. Related to Figure 1. (A)-(C) Young (8-12 weeks) and aged (20-24 months) mice were exposed to single sub-lethal dose of LPS and peripheral blood (A) myeloid cell and (B) granulocyte (C) T cell, (D) B cell, frequencies were measured by flow cytometry at the indicated time points after LPS exposure (n = 4-14 per group). (E)-(F) Mice were harvested 2-3 weeks after the second LPS injection. (E) Splenic CD11b+ cell and CD3e+ cell frequencies are shown. (n=4 per group) (F) The frequencies of different bone marrow progenitor cells are shown. LT-HSCs were gated using the markers Lineage-cKit+Sca1+CD150+CD48-, ST-HSCs using the markers Lineage-cKit+Sca1+CD150-CD48-, MPPs using the markers Lineage-cKit+Sca1+CD150-CD48+ and LSKs using the markers Lineage-cKit+Sca1+ (n=5-7 per group). Data represent at least two independent experiments and are presented as mean ± SEM. * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$ using 2way ANOVA (A-D), or T test (E,F).

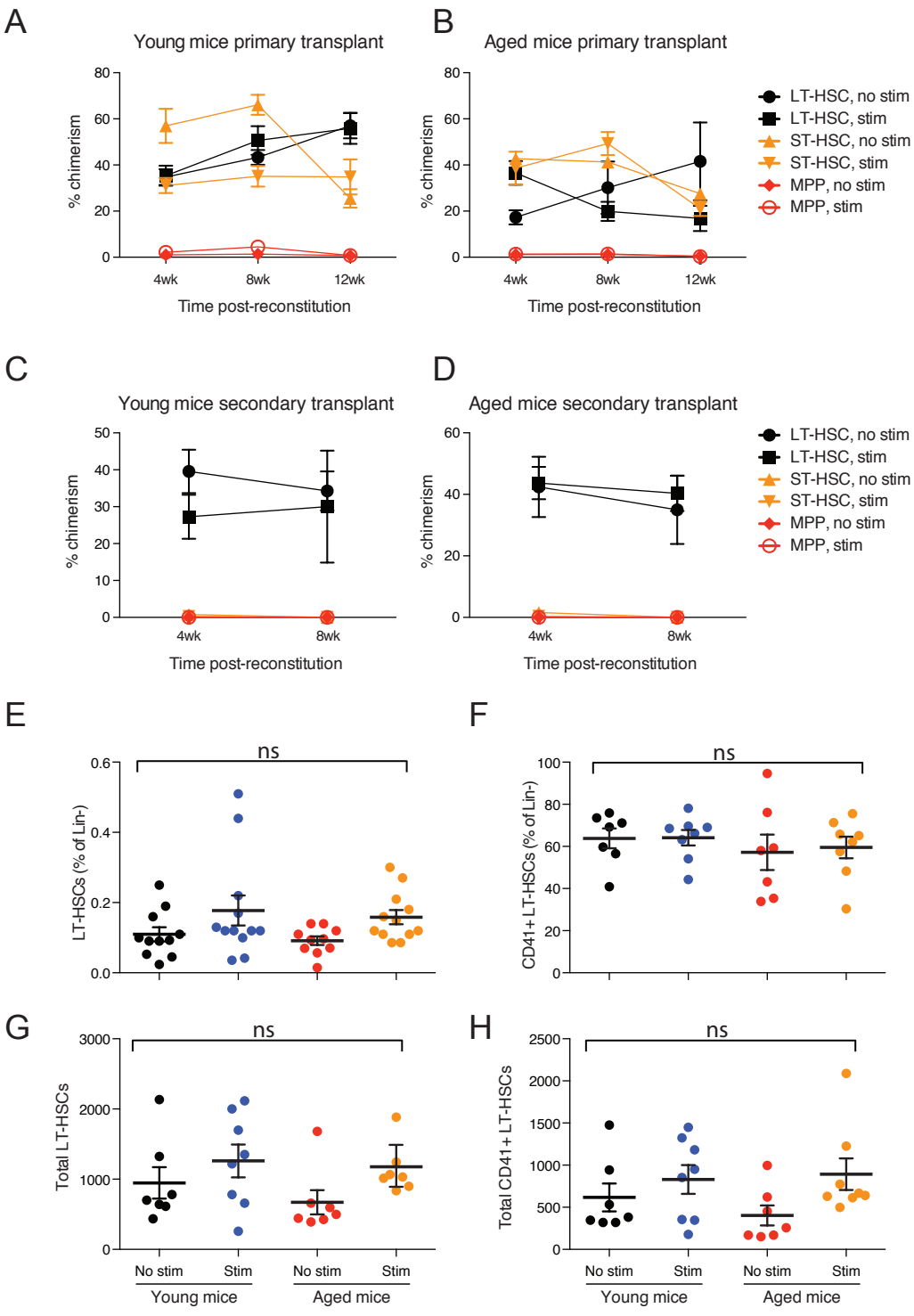

Figure S2. Transplantation of LT-HSCs, ST-HSCs and MPPs from young and aged mice, both with and without stimulation, demonstrates only LT-HSCs have long term reconstitution potential. Related to Figure 1. CD45.2 HSPCs were sorted as described in Figure 2 and treated with or without LPS/Pam3csk4 in vitro prior to transplantation into lethally irradiated CD45.1 recipient mice along with CD45.1 bone marrow helper cells. (A)-(D) These panels demonstrate that LT-HSCs from both (A) young and (B) aged mice, had the ability to maintain long term reconstitution even when stimulated in vitro prior to transplantation. All MPPs failed to maintain long term hematopoietic output. (C)-(D) Secondary transplantation was performed using bone marrow cells obtained from mice in parts (A) and (B). Only LT-HSCs were able to maintain long term reconstitution during secondary transplantation. This was the case for both (D) young and (E) aged LT-HSCs, and both initially unstimulated and stimulated LT-HSCs. (E)-(H) The bone marrow compartment for all mice was analyzed 3 months post-reconstitution for the (E) frequency and (G) total number of LT-HSCs, as well as the (F) frequency and (H) total number of CD41+ LT-HSC subsets. Data represent at least two independent experiments (n=7-12 per group) and are presented as mean ± SEM and analyzed using 1way ANOVA (E-H).

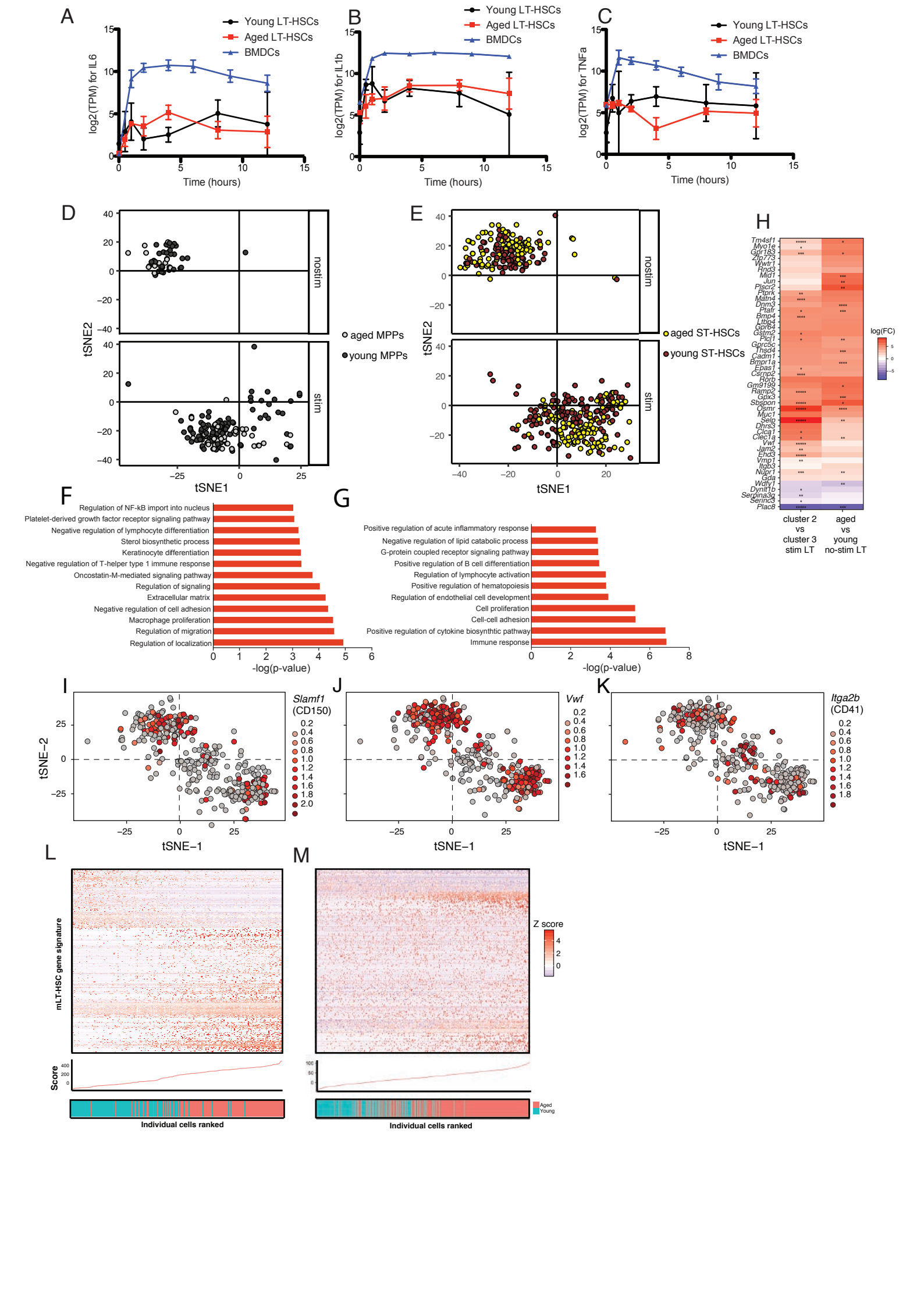
A
Young LT-HSCs
Aged LT-HSCs
BMDCs
log2(TPM) for IL6
Time (hours)
B
log2(TPM) for IL1b
C
log2(TPM) for TNFa
D
nostim
stim
tSNE2
tSNE1
aged MPPs
young MPPs
E
aged ST-HSCs
young ST-HSCs
H
log(FC)
cluster 2 vs cluster 3 stim LT
aged vs young no-stim LT
F
Regulation of NF-kB import into nucleus
Platelet-derived growth factor receptor signaling pathway
Negative regulation of lymphocyte differentiation
Sterol biosynthetic process
Keratinocyte differentiation
Negative regulation of T-helper type 1 immune response
Oncostatin-M-mediated signaling pathway
Regulation of signaling
Extracellular matrix
Negative regulation of cell adhesion
Macrophage proliferation
Regulation of migration
Regulation of localization
-log(p-value)
G
Positive regulation of acute inflammatory response
Negative regulation of lipid catabolic process
G-protein coupled receptor signaling pathway
Positive regulation of B cell differentiation
Regulation of lymphocyte activation
Positive regulation of hematopoiesis
Regulation of endothelial cell development
Cell proliferation
Cell-cell adhesion
Positive regulation of cytokine biosynthetic pathway
Immune response
I
Slamf1 (CD150)
tSNE−2
tSNE−1
J
Vwf
K
Itga2b (CD41)
L
mLT-HSC gene signature
Score
Individual cells ranked
M
Z score
Aged
Young

Figure S3. Inflammatory challenge HSPCs in vitro leads to induction of inflammatory cytokines and reveals a myeloid biased gene signature in LT-HSCs. Related to Figures 2, 3. (A)-(E) LT-HSCs from young and aged mice were exposed to LPS and Pam3CSK4 in vitro for the indicated time and subjected to bulk RNA-sequencing. Shown are the log2(TPM) values for (A) IL6, (B) IL1β and (C) TNFα over time along with values for BMDCs obtained from (Jovanovic et al., 2015). (D)-(L) LT-HSCs, ST-HSCs and MPPs from young and aged mice were stimulated with LPS and Pam3CSK4 and sorted for single-cell RNA-sequencing. (D)-(E) Single-cell t-SNE plots (as in Figure 3) showing (D) MPPs and (E) ST-HSCs among unstimulated and stimulated cells, and color coded by mouse age. Gene Ontology analysis for all genes differentially upregulated (F) or downregulated (G) in LT-HSCs in cluster 3 vs cluster 2. (H) A gene signature defined in unstimulated LT-HSCs based on differentially expressed genes in stimulated LT-HSCs. Comparably changed genes between unstimulated aged vs young were isolated and used to define this preliminary signature, which was later used to initialize a k-means clustering approach. (I)-(K) t-SNE plots of all LT-HSCs analyzed by single-cell RNA-seq showing the mRNA expression levels, color coded for log2(TPM), of (I) CD150, (J) Vwf and (K) CD41 in each individual cell. (L) A gene signature learned from stimulated LT-HSCs shows the underlying heterogeneity among stimulated LT-HSCs. Heat-map demonstrates the expression of all genes in the inferred gene signature for each single stimulated LT-HSC. The panels below shows a score for each cell based on the expression of genes in this gene signature. The color-coded bar at the bottom shows the age of the mouse of origin. (M) The same signature in Figure 3G was applied to an independent data set of unstimulated young and aged LT-HSCs (Kowalczyk et al., 2015). Data represent at least two independent experiments (n=9) and are presented as mean ± SEM.

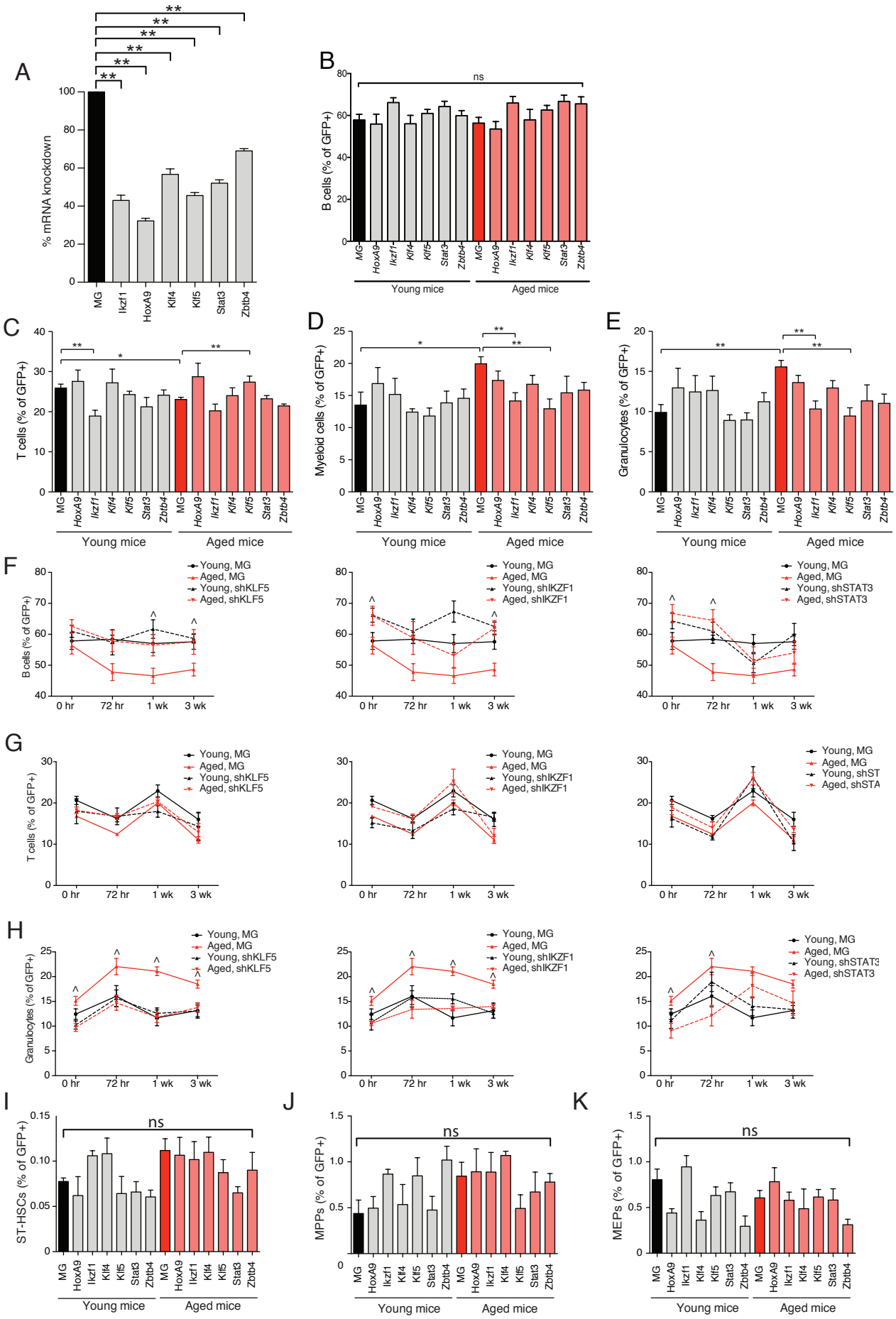
A
% mRNA knockdown
MG
Ikzf1
HoxA9
Klf4
Klf5
Stat3
Zbtb4
B
B cells (% of GFP+)
ns
Young mice
Aged mice
C
T cells (% of GFP+)
D
Myeloid cells (% of GFP+)
E
Granulocytes (% of GFP+)
F
B cells (% of GFP+)
Young, MG
Aged, MG
Young, shKLF5
Aged, shKLF5
Young, shIKZF1
Aged, shIKZF1
Young, shSTAT3
Aged, shSTAT3
0 hr
72 hr
1 wk
3 wk
G
T cells (% of GFP+)
H
Granulocytes (% of GFP+)
I
ST-HSCs (% of GFP+)
J
MPPs (% of GFP+)
K
MEPs (% of GFP+)

Figure S4. Klf5, Ikzf1 and Stat3 knock down regulate myeloid biased LT-HSCs in steady state and in response inflammatory signals. Related to Figures 3, 4. Bone marrow cells from young and aged mice were transduced with constructs to knock-down the indicated transcription factors (TFs). Cells were subsequently reconstituted into lethally irradiated young C57BL/6 recipient mice. (A) Knockdown efficiency of shRNA constructs for each TF analyzed using qPCR. (B)-(E) relative frequencies of (B) B cells, (C) T cells, (D) myeloid cells, and (E) granulocytes in peripheral blood of shRNA transplanted mice. (F)-(H) These mice were subsequently challenged at 4 months post-reconstitution with a single sub-lethal dose of LPS and peripheral blood immune cells were tracked over time by flow cytometry. For each mouse with a knockdown of a TF in the bone marrow compartment, shown are the frequency of peripheral blood (F) CD19+, (G) CD3e+, and (H) Gr-1+ cells over a time-course of 3 weeks after LPS challenge. (I)-(K) Mice were subsequently harvested and analyzed for progenitor populations in the bone marrow compartment. Shown are the bone marrow frequencies of (I) ST-HSCs, (J) MPPs and (K) MEPs in the bone marrow of these mice among GFP+ cells. Data represent two independent experiments (n=8-10 mice per group) and are presented as mean ± SEM. (A-E, I-K) * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$. P-values was corrected for multiple hypothesis testing by Bonferroni's method. (F-H) ^ denotes $p<0.05$ for Aged shRNA vs. Aged MG, v denotes $p<0.05$ for aged shRNA vs. young MG using two way ANOVA.

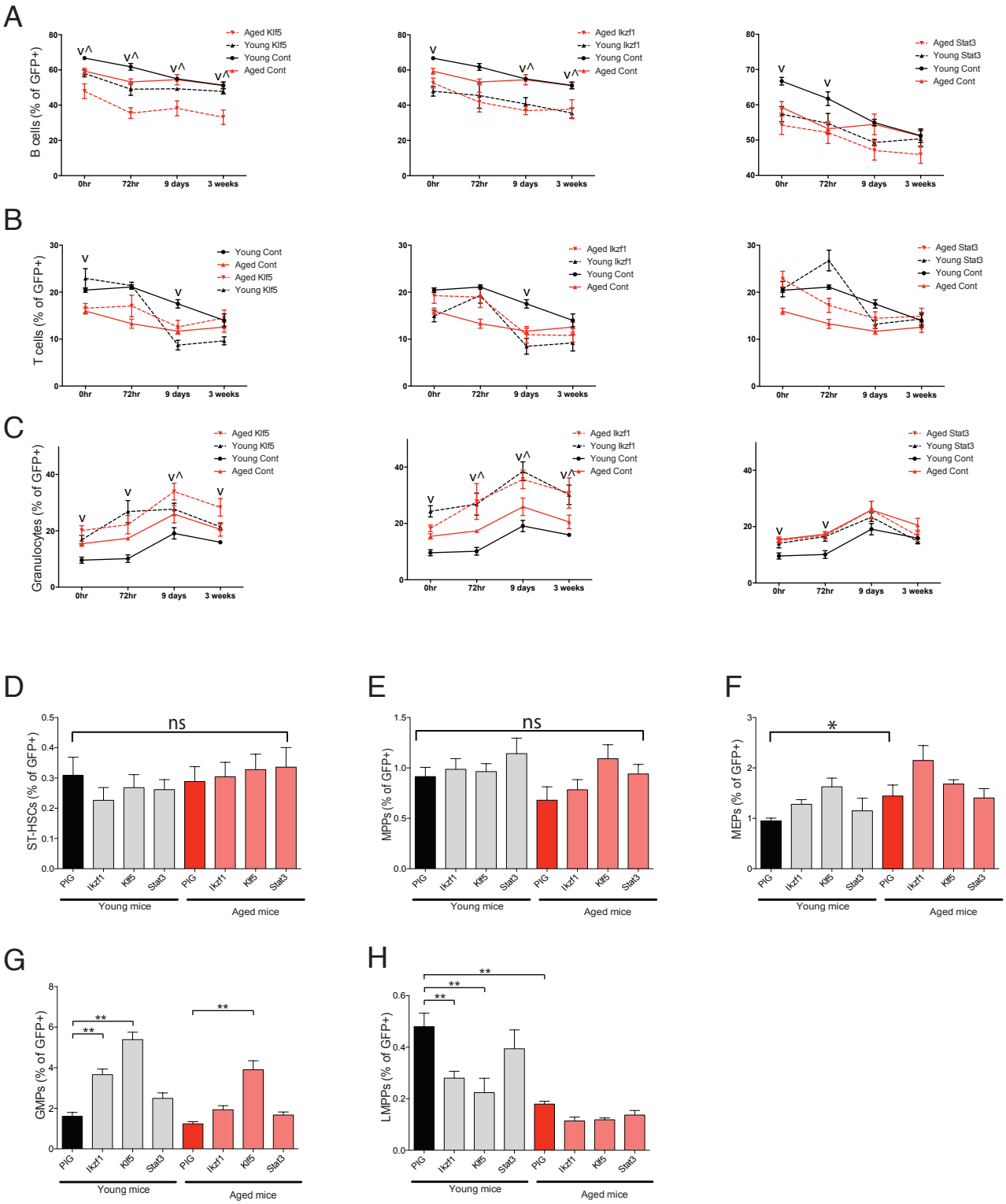

Figure S5. Klf5, Ikzf1 and Stat3 over expression regulate myeloid biased LT-HSCs in steady state and in response inflammatory signals. Related to Figure 4. Bone marrow cells from young (8-12 weeks) and aged (20-24 months) C57BL/6 mice were transduced with constructs to over express Klf5,Ikzf1, and Stat3. These cells were subsequently reconstituted into lethally irradiated young C57BL/6 recipient mice. (A)-(C) These mice were subsequently challenged with a single sub-lethal dose of LPS and peripheral blood immune cells were tracked over time by flow cytometry. For each mouse overexpressing one of the transcription factor in the bone marrow compartment, shown are the frequency of peripheral blood (A) CD19+, (B) CD3e+, and (C) Gr-1+ cells over a time-course of 3 weeks after the LPS challenge. (D)-(H) Mice were subsequently harvested and analyzed for progenitor populations in the bone marrow compartment. Shown are the bone marrow frequencies of (D) ST-HSCs, (E) MPPs, (F) MEPs, (G) GMPs, and (H) LMPPs in the bone marrow of these mice among GFP+ cells. Data represent at least two independent experiments (n=8-10 mice per group) and are presented as mean ± SEM. (D-F) * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$. (A-C) ^ denotes $p<0.05$ for aged overexpression vs. aged control, v denotes $p<0.05$ for aged overexpression vs. young control using two way ANOVA.

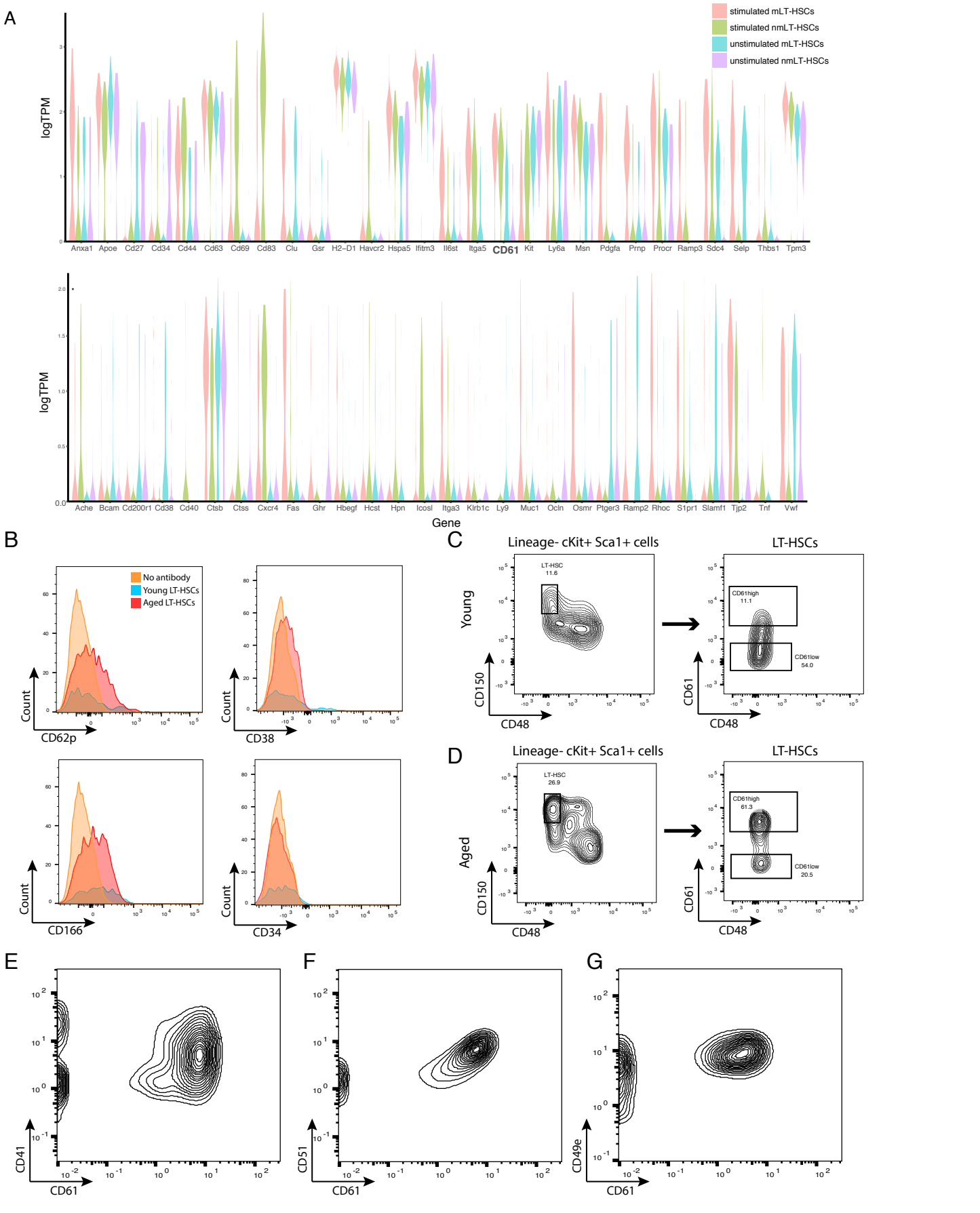

Figure S6. Putative surface markers for the isolation of mLT-HSCs. Related to Figure 5. (A) LT-HSCs from 5 young and 4 aged mice were stimulated with LPS and Pam3CSK4 and sorted for scRNA-seq. Shown are the log2(TPM) values for several genes for which there are commercially available flow cytometry antibodies to detect their surface expression. (B) Surface expression of several putative markers for mLT-HSCs, including CD62p, CD166, CD38 and CD34. (C)-(D) Representative flow cytometry plots showing the sorting of CD61+ and CD61- LT-HSCs from (C) young and (D) aged mice. (E)-(F) Representative flow cytometry plots of aged LT-HSCs stained for CD61 and (E) CD41, (F) CD51, and (G) CD49e.

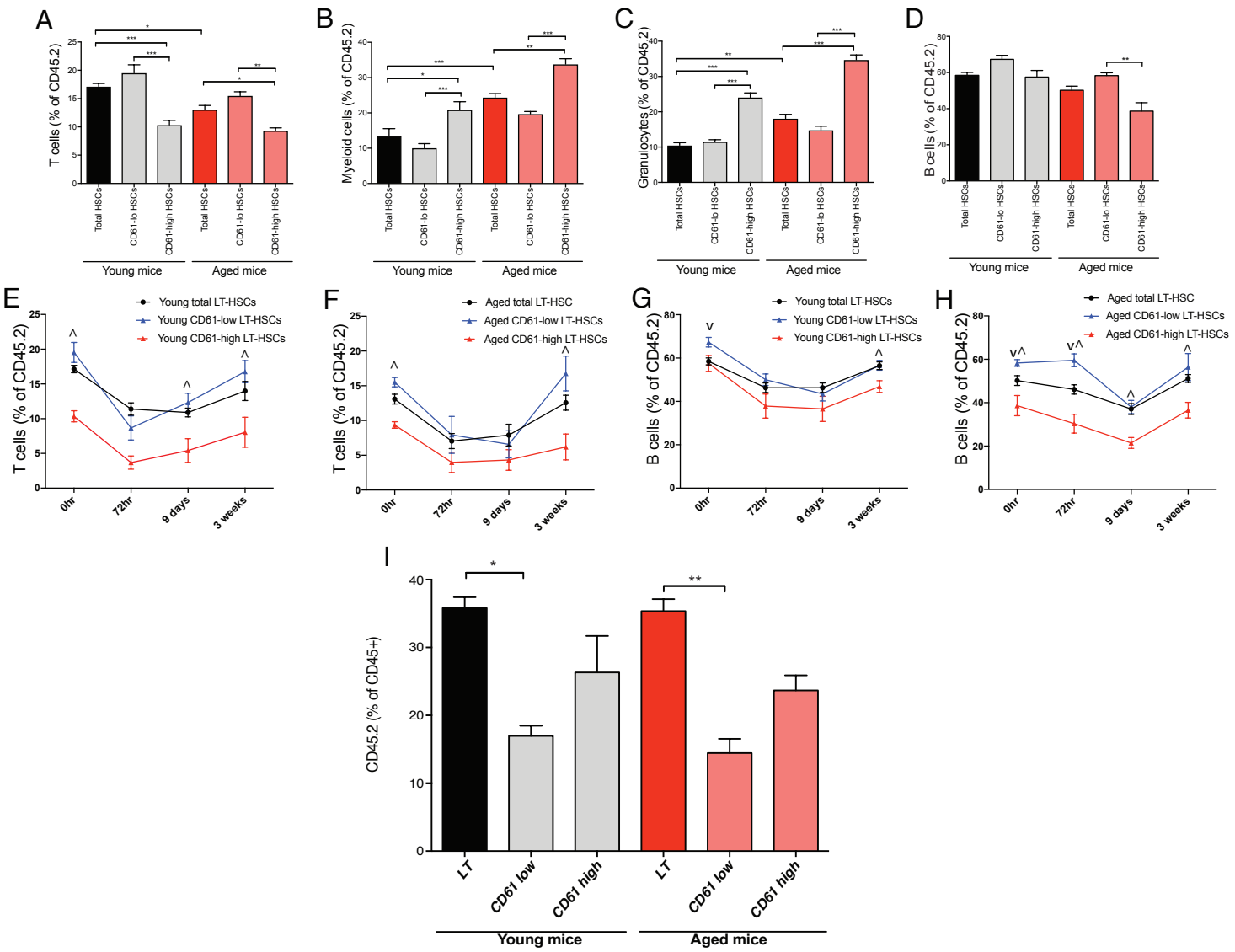

Figure S7. High CD61 expression on LT-HSCs is a marker for mLT-HSCs. Related to Figure 5. (A)-(H) Total LT-HSCs, and those with elevated (CD61-high) and decreased (CD61-low) expression of CD61, from aged and young mice were transplanted into lethally irradiated young C57BL/6 recipient mice. (A)-(D) relative frequencies of (A) CD3e+ cells, (B) CD11b+ cells, (C) Gr1+ cells, and (D) CD19+ cells in peripheral blood of transplanted mice three months after reconstitution. Transplanted mice were subsequently challenged with a single dose of LPS as in Figure 1. Shown are the 3 week time course expression of peripheral blood CD3e+ cell frequencies from mice transplanted with (E) young and (F) aged donor LT-HSCs, and the peripheral blood CD19+ frequencies from mice transplanted with (G) young and (H) aged donor LT-HSCs. (I) reconstitution efficiency of CD61 high and low LT-HSCs was quantified 3 months after transplant using flow cytometry. Shown as percentage of CD45+ cells. Data represent at least two independent experiments (n=7-12 per group) and are presented as mean ± SEM. ^ denotes $p<0.05$ for CD61-high vs. control, vdenotes $p<0.05$ for CD61-low vs. control using two way ANOVA. * denotes $p < 0.05$, ** denotes $p < 0.01$ and *** denotes $p < 0.001$. (A-D,I) P-values was corrected for multiple hypothesis testing by Bonferroni's method. (E-H) ^ denotes $p<0.05$ for CD61-high vs. control, vdenotes $p<0.05$ for CD61-low vs. control using two way ANOVA.

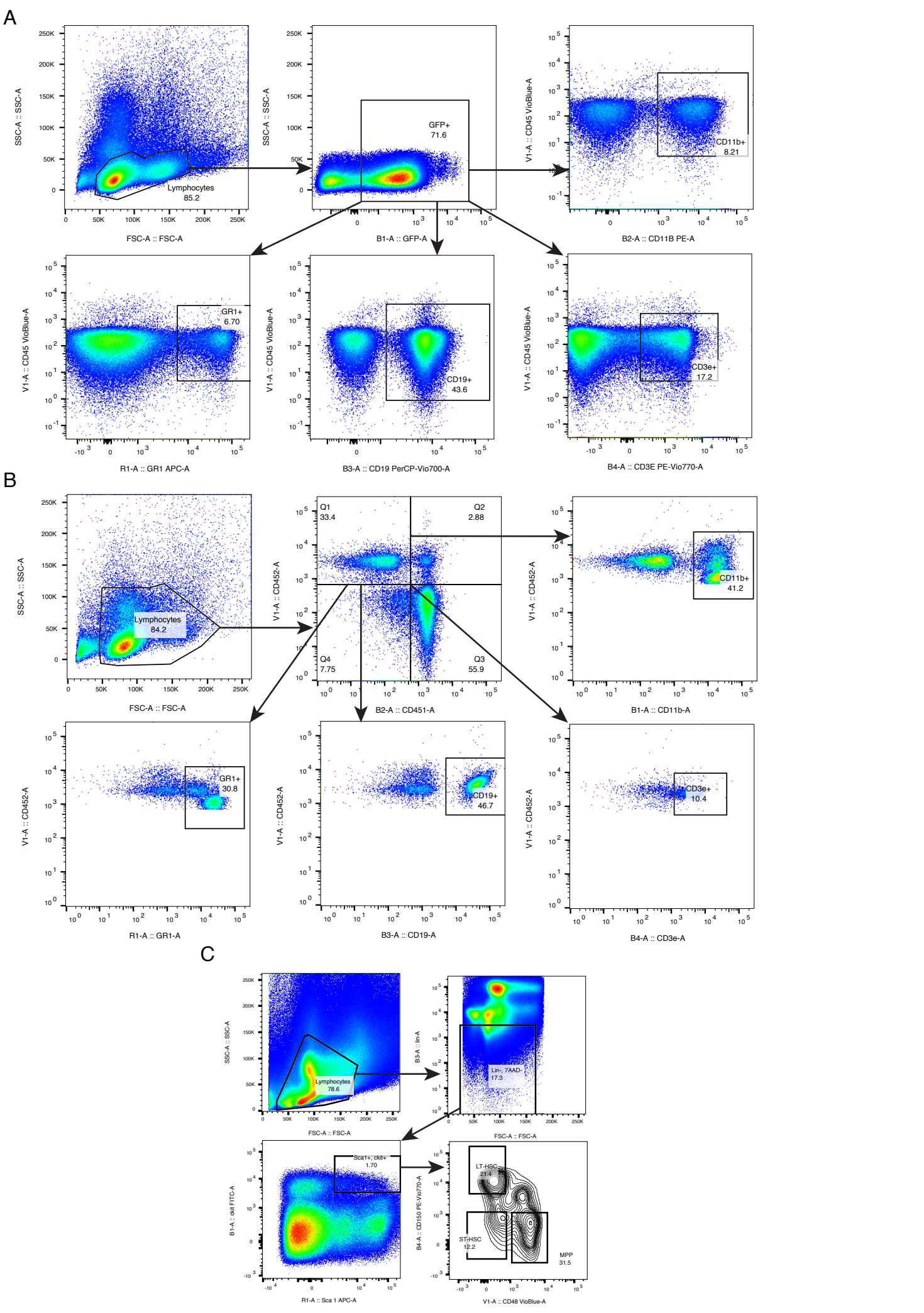
A
SSC-A :: SSC-A
FSC-A :: FSC-A
Lymphocytes
85.2
GFP+
71.6
B1-A :: GFP-A
V1-A :: CD45 VioBlue-A
CD11b+
8.21
B2-A :: CD11B PE-A
GR1+
6.70
R1-A :: GR1 APC-A
CD19+
43.6
B3-A :: CD19 PerCP-Vio700-A
CD3e+
17.2
B4-A :: CD3E PE-Vio770-A
B
Lymphocytes
84.2
V1-A :: CD452-A
Q1
33.4
Q2
2.88
Q4
7.75
Q3
55.9
B2-A :: CD451-A
B1-A :: CD11b-A
CD11b+
41.2
GR1+
30.8
R1-A :: GR1-A
CD19+
46.7
B3-A :: CD19-A
CD3e+
10.4
B4-A :: CD3e-A
C
Lymphocytes
78.6
B3-A :: lin-A
Lin-, 7AAD
17.3
Sca1+, ckit+
1.70
B1-A :: ckit FITC-A
R1-A :: Sca 1 APC-A
LT-HSC
21.4
ST-HSC
12.2
MPP
31.5
B4-A :: CD150 PE-Vio770-A
V1-A :: CD48 VioBlue-A

Figure S8. Flow cytometry gating strategy for: (A) Peripheral blood mature cell analysis for GFP+ CD45+ shRNA/overexpression reconstitution experiments (related to Figures 4, S4, S5). (B) Peripheral blood mature cell analysis for CD45.2+ cells from CD61 high/low reconstitution experiments (related to Figures 5, S6, S7).
(C) LT-HSCs, ST-HSCs, MPPs from young and aged mice, as well as all reconstitution experiments (related to Figures 2-6, S1-7).